Laboratory for Conservation Genetics, Leipzig

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Laboratory Protocol

Protocol number: 3
Protocol description: Fecal DNA Extraction
Original reference: Morin, Chambers, Boesch & Vigilant (2001); Mol Ecol 10:1835-1844.
Original entry: Amy Roeder 31 October 2001
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Required materials:
1. QIAGEN QIAamp DNA Stool Mini Kit (cat. no. 51504)
2. 4x 2ml tubes
3. 4x collection tubes (provided in kit)
4. 1x 1.5ml tubes
5. 100% EtOH
6. carrier RNA (Poly(A) carrier, Roche, cat. no. 0108626)

Required equipment:
1. Microfuge
2. Heatblock/shaker

Protocol
1. Set heatblock to 70°C
2. Make sure that buffers ASL and AL are not precipitated, dissolve soln. at 70°C if necessary
3. Add 100 mg desiccated feces or 250 mg of fresh feces to a 2 ml tube
4. Add 1.6 ml ASL, vortex very well, and soak for 1 h at RT (fresh feces) or 2-72 h (desiccated feces) vortex occasionally while soaking.
5. Centrifuge full speed for 3 min to pellet feces
6. Transfer 1.4 ml of the supernatant into a new 2 ml tube, discard the pellet
7. Add 1 InhibitEX tablet to each sample and vortex vigorously until the tablet is completely suspended
8. Incubate the suspension for a few minutes at room temperature
9. Centrifuge samples at full speed for 10 min
10. Transfer the supernatant into a 2 ml tube
11. Centrifuge the pellet at full speed for 3 min.
12. Transfer the supernatant into the tube from step 10, discard the pellet (you need 600µl of supernatant for step 15 (steps 11 and 12 may be repeated)
13. Centrifuge the supernatant at full speed for 6 min.
14. Pipet 25 µl proteinase K (provided in kit) into a new 2 ml tube
15. Transfer 600 µl supernatant (avoid the white precipitate) from step 13 to the 2ml-tube containing proteinase K
16. Add 600 µl of AL and vortex immediately (15 sec.)
17. Incubate at 70°C for 10 min (go to this step directly after vortexing)
18. Add 4 µl of carrier RNA and vortex immediately
19. Add 600 µl of 100% EtOH to the lysate and mix by vortexing

20. Carefully apply 600 µl of solution from step 19 to a QIAamp spin column
21. Centrifuge at full speed for 2 min, place the spin column in a new 2 ml collection tube, discard the tube containing the filtrate
22. Apply a second aliquot of 600 µl lysate and centrifuge at full speed for 2 min, place the spin column in a new 2 ml collection tube and discard the filtrate
23. Apply the last aliquot of lysate (600 µl) and centrifuge at full speed for 2 min, place the spin column in a new 2 ml collection tube and discard the filtrate
24. Wash the column with 500 µl AW1, centrifuge at full speed for 2 min, discard the filtrate and place column in a new collection tube
25. wash the column with 500 µl buffer AW2, centrifuge at full speed for 6 min, discard the collection tube with filtrate
26. optional: place the spin column in a new collection tube and centrifuge at full speed for 2 min, discard the collection tube containing filtrate
27. transfer the spin column into a labeled 1.5 ml tube and pipet 200 µl buffer AE directly onto the membrane
28. incubate for 20-30 min at RT and then centrifuge at full speed for 2 min to elute the DNA